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ROY WALDO MINER

QUANTITATIVE RELATIONS BETWEEN LIVER MITOCHONDRIA METABOLISM AND TOTAL BODY WEIGHT IN MAMMALS

BY
ROBERT E. SMITH

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Department of Physiology, University of California Medical School,
Los Angeles, Calif.; and Neurology Research Unit,
Veterans Administration Center, Los Angeles, Calif.

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BY

ROBERT E. SMITH**

Introduction

The present problem arose from a desire to establish, at the cellular level, a biological basis for the relation between cell metabolism and the total body size of the organism. In 1932, Kleiber[1] and, independently, Brody and Proctor[2] took a definitive step in relating the heat production of mammals to their body size by adopting, as their reference unit, a power function of body weight explicitly derived from the data. They showed, in adult mammals ranging from the mouse to the elephant, that the plot of the logarithm of metabolism per animal and unit time against the logarithm of the body weight was best fitted by a straight line of slope about 0.73. On this basis they could write the appropriate equation of similitude; *i.e.*

$$H = aW^{0.73} \tag{1}$$

stating that total body heat production, H (or oxygen utilization), was proportional to the 0.73 power of body weight, W.

Recent theoretical and empirical studies (*cf.* Hemmingsen,[4] Behnke,[5] von Schelling[6]) on body geometry, allometry, and metabolic exchange have indicated that, in mammals, the proper values of both coefficient and exponent (0.73), as well as of minimum weight of the function, aW^k, may be uniquely specified within remarkably narrow limits. Other work, however, extending through the extremes of size in the animal kingdom, supports the thesis that the exponent will have approximately the above value at the upper and lower extremes of sizes but a higher value, *ca.* 0.95, among certain groups of intermediate size (Zeuthen[3]). In any event, there has been reason to feel that the relationship expressed by EQUATION No. 1 may have the properties of a general law. Its implications have therefore been under study by a series of investigators over the past two decades, and the attack has carried into several levels of structural and functional complexity.

For appreciation of what follows, it is well to note that EQUATION No. 1 can be transformed into one in which the exponent is negative instead of positive merely by dividing both sides of the equation by weight. Thus, for regression of total metabolism *per unit weight* on total body weight, Equation No. 1 becomes

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$$\frac{H}{W} = aW^{(0.73-1)} = aW^{-0.27} = aW^{b} \tag{2}$$

in which H/W may be referred to as the "weight-specific" metabolism. For clarity, the exponent in this form will be designated as b. It is clear, however, that denoting the exponent of Equation No. 1 as k, $b = (k-1)$ is possible only where we may properly divide through by $W^{1.0}$. For the general case, $b = (k-m)$ where m is often less than unity. Thus for QO_2 of a given organ, the total oxygen uptake by the organ will be the product of its (dry) weight, W_i, times the QO_2. As a function of body weight, we have empirically

$$W_i = a_1 W^{m_i} \tag{3}$$

where m_i is here the allometric constant for the regression of the log weight of the given (ith) organ on log total body weight. Empirically also,

$$QO_2 = a_2 W^{b_i} \tag{4}$$

and the product, giving total oxygen uptake per organ and hour, is

$$\phi O_2 = QO_2 \; W_i = a_2 \, a_1 \, W^{k_i} = a_3 W^{(b_i + m_i)}$$

whence

$$\phi O_2 = a_3 W^{k} \tag{5}$$

It is of general interest to note that Equation No. 1 actually represents a biological summation in which

$$H = \sum_{i=1}^{n} (\phi O_2)_i = \sum_{1}^{n} a_i W^{k_i} = aW^{k} ,$$

where $k = f(k_i)$ such that the observed exponent, *e.g.* 0.73, is some resultant or weighted mean of a spectrum of k_i values represented by the various organ systems contributing to the whole. The important fact to note here, however, is that numerical identity in either the k_i or the b_i values with those of the whole animal or another organ system may be coincidental unless the m values are also accounted for. The converse is equally pertinent, namely that with knowledge of and due correction for the allometric constant, m, a coincidence of k values actually may be achieved despite apparent discrepancies in the measured values of b. Certain corrections of the latter sort have been employed in the present paper with interesting results.

Studies designed to explore the fundamental nature of the relationship between total metabolism and body size have now been extended to nearly all levels of structural and biochemical organization. These are reviewed briefly in order to complete the framework for the present investigation.

In mammals, Brody and his associates[7] showed the same (~0.70) power function to apply to total endogenous nitrogen and neutral sulfur and later, Drabkin[8] gave added confirmation with respect to total cytochrome c content in rat, dog, man, and horse.

Metabolic studies at the tissue level, despite a certain confusion of interpretation, appear generally to reinforce the basic conclusion derived from total metabolism studies. Thus for mammalian liver, Kleiber[9] and Weymouth, Field, and Kleiber[10] obtained QO_2 data on rats, rabbits, and sheep giving $QO_2 = a'W^{-0.228 \pm .017}$, which compared favorably with the exponent of total metabolism when expressed per unit weight for these species; *i.e.*,

$$\frac{H}{W} = aW^{-0.243}$$

Data from a more recent and detailed study by Krebs[11] on a series of five tissues in nine mammalian species give, with similar treatment, a regression for QO_2 of liver versus body weight of -0.115; except for spleen, the slopes of other tissues are somewhat nearer zero.

At the molecular level, Rosenthal and Drabkin[12] were able to show that the concentration of cytochrome *c* in kidney cortex declined with body weight, giving a regression of -0.278. Similar conclusions were also drawn for cytochrome oxidase (Drabkin[13, 14]) and, by inference, succinoxidase. The latter, as well as malic dehydrogenase, has been assayed recently in liver, heart, kidney, and brain for the mouse, rat, and cow by Fried and Tipton.[15] The data of these investigators indicate concentrations decreasing significantly with increasing body size. A logarithmic plot made of these data gives slopes all lying between -0.10 and -0.15, except for malic dehydrogenase in liver and kidney. Kunkel and Campbell[16] have extended the interspecific allometric pattern for cytochrome oxidase, having determined both enzyme concentration and total organ amounts in several tissues of mouse, rat, and dog. The regressions of 0.70 and 0.80 for enzyme amount in kidney and liver respectively are consistent with the pattern cited above for cytochrome c, and the results on concentration in skeletal muscle ($b = -0.24$) in rat are shown to coincide with certain published results on total metabolic exchange as a function of body size.

Localization within the mitochondria of the intracellular enzyme systems subserving aerobic oxidation derives from a long history of convergence wherein the particulate, nonsoluble cell fraction of biochemical experience has been identified with the subcellular structural entity of cytological acquaintance. In the former discipline, Lehninger[17] cites the original findings of Warburg (1913) while, in the latter, Bourne[18] notes that Kingsbury, in 1912, first assigned a respiratory role to the mitochondrion. With this identity now well established, it follows in any case that, at least in a general way, the relative intensity of aerobic respiration in tissues should be proportional to their mitochondrial content, and recent studies by Paul and Sperling[19] on skeletal muscle appear to confirm this (*cf.* also Drabkin[8]). The present work was initiated to characterize the mitochondria in respect to number and amount or to other parameters, to test sufficiently the implication that these would show a relationship to total body size comparable to that already noted in both total metabolism and total enzyme content.

Materials and Methods

Livers from percussion-stunned and/or decapitated adult rats, rabbits, sheep, and steers were rapidly chilled and exsanguinated by perfusion with cold (0 to 5° C.) KCl (0.16M) either *in vivo* or within 5 to 15 minutes of killing (steers and sheep). All animals were fasted 15 to 18 hours prior to use. After pressing 10 to 12 gm. liver aliquots through the grid (0.9 mm. holes) of a garlic press, the press pulp was added to 3 volumes of KCl, and homogenates were made in a 50 ml. Potter-Elvehjem type smooth glass grinder with tephlon pestle by 20 up-down strokes at rotation speed of *ca.* 700 rpm. The resulting brei was transferred quantitatively and made up to 50 ml. with additional KCl. From this, an aliquot (H) of 8 or 10 ml. was removed for direct use in respiration trials, and the remaining amount of 40 ml. was split into two 50 ml. lusteroid tubes for centrifugation, using a refrigerated centrifuge (model PR-1, with 8-place horizontal head, International Equipment Co., Boston, Mass.). After 20 minutes (10 minutes in rat) at 2600 rpm. (1500 G) plus 75 seconds to steady velocity, the supernatant was removed (*S* fraction), and the sediment of each tube was resuspended in 10 ml. of 5.75 per cent mannitol, using Difco d-mannitol in distilled water, filtered through No. 50 filter paper (Leuthardt and Müller[22]). From a second spinning like the first, the supernatant was added to the first, and the total *S* fraction was made up to known volume (100 ml.) with mannitol. The sediments were each resuspended in 10 ml. mannitol by drawing three times into a 10 ml. syringe. The centrifugation schedule for this mix was varied from three minutes at 2600 rpm. for rabbits to two and one-fourth minutes at 2600 rpm. for the other species (plus 75 seconds to maximum speed in all trials). This served largely to sediment the unbroken cells and nuclei, and the mitochondria were drawn off in the supernatant (*M* fraction). This schedule was repeated after resuspension of the sediment as before, and the second supernatant was added to the previous one. This total was then diluted to 40 or 50 ml. with mannitol to give the final volume of the *M* fraction. At this point, the sediments were combined and brought up with mannitol to 50 ml. volume (*N* fraction).

Dry weights of all materials were obtained as constant weights at 90° C. Suitable aliquots of fresh liver were weighed wet in closed weighing vessels and then dried. The press residue of collagenous debris was dried before weighing. Dry weights of homogenate and fractions were obtained in triplicate or quadruplicate on 5 ml. samples. Prior to weighing, each of these were placed in 8/32-inch diameter Visking cellulose tubing and dialyzed for 24 hours in two changes of 1000 volume excess of distilled water, stirred at 3° C. The respective samples were then transferred quantitatively to tared vials, dried, and weighed.

Mitochondrial counts (cf. Allard *et al.*[23]; Shelton *et al.*[24]) were obtained on each fraction by diluting 1:100 in cold distilled water or mannitol solution, and viewing with dark *M* phase contrast at 970 times in a Petroff-Hausser bacterial counting chamber. Dilutions were made in a red cell counting pipette, and counts were begun within one minute after the chamber was filled. A total of 50 of the smallest squares in 5 areas of the chamber were counted. Usually three separate dilutions and counts were made and averaged for the *M* fraction, two for *N* fraction, and only one for *S* fraction. The last of these was usually nearly void of mitochondria; the *N* fraction contained a fairly high number for various reasons. The counts for each fraction were corrected by the appropriate dilution and gravimetric factors, and were finally added together to give the total number of mitochondria present in the original liver sample. Several checks of counts summated from the respective fractions against that of the original homogenate indicated satisfactory agreement. The homogenate was not regularly counted, however, since clumping of the mitochondria usually occurs in the presence of electrolyte.

Oxygen utilization of homogenates and fractions was determined by the direct Warburg technique at 37° C. One ml. of the respective fractions was initially added to a standard medium including substrate and other ingredients to give a final volume of 3.0 ml. in 15 ml. flasks. The final concentrations of components in the medium were: $MgCl_2$, 0.002M; d-mannitol, 0.183M;* ATP, 0.00067M; AMP (adenosine-5 phosphate), 0.0067M; cytochrome c, 5×10^{-5} M; alpha-ketoglutarate, 0.0033M; phosphate buffer, 0.02 M in phosphate and 0.069 M in potassium; final pH, 7.2. A basic medium, containing phosphate buffer, $MgCl_2$, and mannitol, was made up in 500 ml. quantities. ATP and AMP (as sodium salts),

*The final concentrations of mannitol in the respiration system varied according to the fraction as follows: homogenate, 0.183 M; soluble, 0.23 M; nuclear and mitochondrial, 0.29 M. Final KCl concentration in homogenate was 0.15 M; that in nuclear and in mitochondrial fractions, 0.07 M.

cytochrome *c*, and alpha-ketoglutarate were weighed out fresh each day and dissolved in an appropriate volume of medium. The solution was then neutralized with 10 per cent KOH. Center wells of the flasks were charged with 10 per cent KOH and filter papers. The flasks were oxygenated, and readings were taken at 5-minute intervals, beginning at 20 minutes after introduction of the brei. The succeeding 30 minutes were used as the basis of computations on oxygen uptake. The average time from animal killing to first reading was 120 minutes; however, no consistent effects associated with shorter and longer times up to 160 minutes were noted.

From preliminary studies, it was found that the apparent QO_2 increased with the brei concentration up to about 4 mg. dry weight per flask. This dilution effect was excluded by use of brei concentrations in excess of this value throughout the series. As a control measure, two levels of concentration were always employed, *i.e.* 0.5 ml. and 1.0 ml. of brei per flask with only the latter being used for computation of the final QO_2. These were not accepted either if the dry weight proved less than the specified minimum.

Choice of the particular medium employed was predicated on the assumptions (1) that preservation of the structural integrity of the mitochondrion was of primary concern, and (2) that the addition of cytochrome c would exclude at least this agent as the limiting factor in the system. Regarding assumption No. 1, it is evident from Raaflaub's recent studies[25] that the structural integrity of mitochondria *in vitro* is improved by lowered pH and by the addition of ATP. This author has shown also that, of the substrates tested, succinate was clearly the most disruptive of structural integrity.

Besides total body weight, independent variables measured included total liver weight (minus gall bladder, which amounted to about 16 per cent of total liver weight in beef), wet and dry weights of whole liver aliquots, dry weights of fractions and of homogenate, dry weights of press residues, number of mitochondria per ml. of fraction, and oxygen utilization of homogenates and fractions.

Results and Discussion

Technical data are largely summarized in TABLE 1. In particular, small interspecific differences appeared in the amounts of fibrous residue withheld by the initial pressing. Since from ancillary data it is felt that these are not due to differences in organ loci of aliquots, they give some indication of the probable correction values which might be required on *in vitro* exchanges of liver slice among these species. Total yields given on the fractions are based on their dialyzed dry weights, and while their internal consistency is extraordinarily high, the apparent deficit of 10 per cent to 15 per cent in recoveries evidently represents (1) the loss during dialysis of soluble constituents additional to mannitol and salts, and (2) losses incurred (particularly from the *S* fraction) from adherence on the inner walls of the dialysis tubes. For present purposes, however, it is relevant to note that between species the total recoveries do not vary more than about 5 or 6 per cent while, intraspecifically, except in sheep, there are no significant differences between total yields from homogenate as compared with the summated fractions. The exception noted in sheep is reflected further in the comparatively large variance in this species in relative yields of the respective fractions. It may be be that in the sheep, the relative increase observed in the *S* fraction at the expense of the *N* fraction is due to the greater fat content of the cytoplasm and, possibly, also to greater fragility of the nuclei.

TABLE 1

SUMMARY OF DESCRIPTIVE AND MORPHOLOGIC DATA

Datum	*Species and measured value of datum*			
	Rat	*Rabbit*	*Sheep*	*Steer*
Breed	White Wistar	Abbey	Mixed	Hereford
No. of animals	13 - 20	4 - 8	10	8
Age (months)	67 - 80 days	4	6 - 9	24
Total body wt. (kg.)	0.219 ± .0071*	1.608 ± .067(4)** 1.514 ± .068(8)	47.02 ± 2.15	447.7 ± 18.9
Liver wt. (gm., dry)	1.846 ± .063	10.54 ± 1.80(4)	128.95 ± 5.97	1068 ± 65
Liver aliquot:				
Dry wt./wet wt.(%)	17.56	16.44	21.29	17.65
Fibrous residue(%)	4.242 ± .169	6.54 ± .909	4.081 ± 0.764	6.953 ± .354
Liver fractions:				
Total yields (% of pressed pulp)				
Homogenate	91.08 ± 1.35	88.61 ± 1.92	91.29 ± 1.165	86.91 ± 1.41
Sum of fractions	91.77 ± 1.41	87.34 ± 2.05	88.01 ± 1.452	86.03 ± 2.40
Relative yields(%)				
S	42.45 ± 1.08	40.11 ± .92	61.44 ± 1.82	48.76 ± 1.52
M	26.27 ± 0.67	13.55 ± 1.23	14.13 ± 1.08	20.53 ± 1.22
N	31.29 ± 0.93	46.29 ± 1.85	24.38 ± 1.06	30.71 ± 1.73
Mitochondrial data				
No./gm.d.w. liver ($\times 10^{-11}$)	5.149 ± 0.2417	2.960 ± 0.1469	2.448 ± 0.1322	2.234 ± 0.0885
No./ total liver ($\times 10^{-11}$)	9.41 ± .702	30.23 ± 4.55	312.8 ± 14.2	2376 ± 159
Dry mass/mito. (mg. $\times 10^{10}$)	7.21 ± 0.39	7.93 ± 0.84(7)	9.48 ± 0.56	10.79 ± 0.74

*Standard error of estimate.
**Number of rabbits included; eight unless specified.

Mitochondrial frequencies per gram of dry liver decreased progressively from rat to steer though, stepwise, the group differences clearly fail of significance in the decrements from sheep to steer. Conversely, the apparent dry mass of the individual mitochondrion increased progressively with increasing size but only slowly. While the increments between successive groups are not significant by the *t* test, those between rat and sheep, or between either rat or rabbit and steer are reasonably so. Thus the mitochondria are evidently smaller and more numerous in the small than in the large animals.

Oxygen utilization (TABLE 2) of the homogenate and the respective fractions was expressed in terms of the QO_2 and also as the quotient μl O_2/mitochondrion and hour. For the fractions, the latter was computed directly as the oxygen utilized per hour per ml. of the suspension. For the mitochondrial number in homogenate, the summated counts of the fractions were used. The general trend of QO_2 for both homogenate and fractions was toward decreased values with increasing body size. Oxygen utilization per mitochondrion, however, appeared not to vary significantly in this respect.

The interspecific regressions of the respective parameters upon body size were derived from the intraspecific means without regard to variance within the latter. Thus the method of least squares was applied in each case to the four points of a plot of log mean variable versus log mean body weight, each point representing the mean of a given species. Despite the statistical inefficiency of this procedure, it is sufficient to show (TABLE 3) that all but one of the regressions are significantly different from zero.

For purposes of discussion, it is convenient to consider the regressions of TABLE 3 under two general categories. The first examples (1) are essentially *weight specific, i.e.* referred to unit weight of liver and, therefore, in general having negative slopes. Under the second category (2) may be included the regressions of quantities which are referrable to the whole organ and, hence, have positive slopes, usually of the order of 0.6 or greater. For the sake of distinction, these will be referred to as *organ specific.*

Weight specific regressions. In TABLE 3, the slopes of those quantities expressed per unit mass of liver (*i.e.* Y_2, Y_5, Y_7, Y_8, Y_9, and Y_{11}) cluster around a value of about -0.13 or less (-0.115 if QO_2 of the nuclear fraction is ignored). It is suggestive that QO_2 of homogenate (Y_7) has a slope not significantly greater than that (Y_{11}) for oxygen utilized by mitochondria in each gram of liver. A similar parallelism applies to the slopes exhibited by QO_2 of mitochondrial fraction (Y_8) and number of mitochondria per gram of liver (Y_2). Of these four, however,

TABLE 2

OXYGEN UTILIZATION OF HOMOGENATE AND CELL FRACTIONS FROM LIVER OF DIFFERENT SPECIES

Species	*Body weight*	*Homogenate*		*Mitochondrial*		*Nuclear*		*Soluble - micros.*	
	(gm.)	QO_2	$\frac{\mu l\ O_2}{\text{mito. \& hr.}}$***	QO_2	$\frac{\mu l\ O_2}{\text{mito. \& hr.}}$***	QO_2	$\frac{\mu l\ O_2}{\text{mito. \& hr.}}$***	QO_2	$\frac{\mu l\ O_2}{\text{mito. \& hr.}}$***
Rat	219.3	22.08 (1.71)*	37.34 (2.82)	28.20 (1.11)	19.65 (1.16)	28.84 (2.18)	42.77 (6.88)	3.32**	220
Rabbit	1607.5	15.44 (1.03)	42.0 (2.35)	24.43 (1.21)	14.73 (0.37)	20.28 (0.87)	57.35 (5.19)	0	–
Sheep	47.02 × 10^3	7.17 (0.42)	25.28 (1.60)	16.50 (2.12)	15.17 (1.79)	8.25 (0.80)	19.24 (3.11)	1.91	41.7
Steer	447.7 × 10^3	8.23 (0.60)	30.33 (2.71)	14.47 (0.81)	15.38 (0.85)	8.68 (1.38)	32.36 (10.10)	2.10	540

*Standard error of estimate.

**These values were consistently too low for reliable estimate.

***× 10^9.

TABLE 3

INTERSPECIFIC REGRESSIONS OF LOG Y VERSUS LOG BODY WEIGHT

$Y = aW^b$ (W in grams)

Y	a	b*	s_b	$P<$**	$s_{\log y \cdot \log x}$
1. Liver weight dry (gm.)	0.02266	0.8196	.0091	.01	.0669
2. No. mito./gm. liver	74.54×10^{10}	−0.0991	.0116	.02	.0847
3. No. mito./total liver	1.653×10^{10}	0.7216	.0142	.01	.0104
4. Apparent mito. wt. (mg.) (dry)	0.5407×10^{-9}	0.0527	.0003	.001	.0235
5. Wt. (mg.) mito./gm. liver	405.5	−0.0470	.0400	.4	.0859
6. Dry wt. mito./ total liver	8.922	0.7744	.0148	.01	.1086
7. QO_2 Homogenate	44.59	−0.1445	.0143	.01	.1049
8. QO_2 M fraction	46.75	−0.0922	.0027	.01	.0199
9. QO_2 N fraction	70.45	−0.1745	.0137	.01	.3172
10. μl. O_2/mito. x No. mito./liver = μl O_2/liver (M)	334.8	0.6966	.0205	.01	.1505
11. μl. O_2/mito. x No. mito./gm. d.w. liver = μl. O_2 of mito./gm. d.w. liver	15244	−0.1249	.0187	.02	.1374

*The exponents will correspond variously to b, k, or m, as defined in the text.
**Probability that $\beta = 0$, computed from $t = b/s_b$, d.f. = 2.

only the difference between Y_8 and Y_9 appears significant at better than the 5 per cent level.*

Noting that the pairs of similar slopes, *i.e.* for Y_2, Y_8 and Y_7, Y_{11}, are from independently derived quantities, one is led to infer in each case that the QO_2 is a function of mitochondrial amount. The essential question appears to be whether this is a function of mitochondrial number and/or mass, or possibly mitochondrial surface.

The apparent dependence of QO_2 upon mitochondrial number and mass was examined further by statistical studies of the arithmetic regressions and correlations between the variates. It is evident that QO_2

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*The t value for this comparison was computed as:

$$t = \frac{b_1 - b_2}{\sqrt{(Sb_1)^2 + (Sb_2)^2}}$$

with P estimated for 2 degrees of freedom. A more efficient statistic would probably have improved the P value of the difference obtained here as well as with the couplet $(Y_7 - Y_2)$, where the present treatment gave $0.1 > P > 0.05$.

of homogenate should give a regression on QO_2 of mitochondrial fraction of about unity if the mitochondria of the homogenate remained relatively uninfluenced by the association with disrupted cell components. The least squares regression of slope 0.939 ± .081 obtained between these appears to confirm this expectancy. Partial correlation coefficients were obtained between the three variates (*cf.* TABLE 4), *i.e.* number of mitochondria per gm. (1), QO_2 *M* fraction (3), and QO_2 homogenate (4) (*cf.* Hoel,[20]). These indicated that about half of the covariance was accounted for on a basis of number since, where number was held fixed, the correlation between the QO_2 variates ($r_{34.1} = 0.71$) was reduced. If mitochondrial QO_2 were fixed, however, QO_2 of homogenate still correlated significantly with number ($r_{14.3} = 0.76$). Conversely, while the biserial correlation of number versus QO_2 of *M* fraction was fairly high ($r = 0.81$), the partial correlation, $r_{13.4} = -0.124$, was about zero. Biologically, this is understandable when one recalls that the *M* fraction was essentially a pure suspension of mitochondria and that the QO_2 obtained from it was expressed in terms of microliters oxygen per unit of dry mass, *i.e.* in

TABLE 4

SUMMARY OF COEFFICIENTS OF CORRELATION BETWEEN VARIATES*

	Partial correlation for variate held constant				*Biserial correlation coefficient*	*Multiple correlation coefficient*	
	1	2	3	4			
r_{12}			−0.376	−0.197**	−0.642***	$r_{1.24}$	0.92
r_{13}		0.649		−0.124	0.810	$r_{1.23}$	0.812
r_{14}		0.863	0.755		0.916	$r_{4.12}$	0.941
r_{23}	−0.558			x	−0.774	$r_{3.12}$	0.876
r_{24}	−0.550		x		−0.753		
r_{34}	0.712	x			0.913		
$\bar{x}$	325.4	0.881	20.22	12.88			
s	704	1.21	7.49	7.24			

*Variates: (1) Number of mitochondria per gm. liver ($\times 10^{-9}$)
(2) Mass per mitochondrion (mg.$\times 10^{9}$)
(3) QO_2 of M fraction ($\mu l\ O_2$/mg. dry wt./hour)
(4) QO_2 of homogenate

**The partial correlation, $r_{12 \cdot 4}$, *etc.*; x indicates correlation was not estimated.

***Read $r_{12} = -0.642$, *etc.*
$n = 29$
Standard regressions: (*cf.* Snedecor[21]).
$x_3 = 0.5312\ x_1 - 0.4330 x_2$
$x_4 = 0.7358\ x_1 - 0.2808 x_2$

terms of *amount* of mitochondrial substance, irrespective of either number of unit mass of the mitochondrion *per se*.

These conclusions are strengthened by an examination of the partial correlations (TABLE 4) obtained between QO_2 of the M fraction (3) and the respective numbers of mitochondria per gm. (1) and mass per unit mitochondrion (2). Here the values, $r_{13.2} = 0.65$ and $r_{23.1} = -0.56$, while significantly different from zero by the z transformation, are of the same absolute magnitude and so afford no basis for selection in respect to relative association of QO_2 with either parameter. A similar conclusion is supported by reference to the standard regressions of the QO_2 variates against mass and number (TABLE 4). By comparison with the M fraction, the homogenate contained several materials contributing to its dry mass besides mitochondria. Hence, while the total oxygen utilized was determined by mitochondrial amount, the QO_2 was a function of the amount (number times unit mass) of the latter relative to the inactive components also present. In view of these and the foregoing considerations, it thus appears that the parameters, number and unit mass of the mitochondrion, may be treated as truly independent variables only in connection with the homogenate or nuclear fractions.

On this basis, a further examination was then made of the partial correlations (see TABLE 4) between QO_2 of homogenate (4), number of mitochondria (1), and mass per mitochondrion (2). Here it developed that for QO_2 a somewhat stronger partial correlation appeared with number than with unit mass of the mitochondria, *i.e.*, $r_{14.2} = 0.86$, compared with $r_{24.1} = -0.55$. Transformation of these variables into z values (*cf.* Hoel[20]) with application of the t test, indicates the difference between them to be highly significant, irrespective of the algebraic signs. It is concluded, therefore, that QO_2 of the homogenate is more closely dependent on the number of mitochondria present than on the mass per mitochondrion.

Organ specific regressions. Those systems for which the k_i values (as defined in the introduction) are directly given by the regression of log variate on log body weight include (TABLE 3) those in which the parameters are expressed as total amounts per organ, *i.e.*, Y_3, Y_6, Y_{10} It will be noted that each of these derives from multiplying liver weight by one or another of the weight specific variates discussed earlier. Hence each of these contains, directly or indirectly, the slope of log liver weight on log body weight ($m = 0.8196$) given as Y_1 in the table. While the slopes of all of the variates enumerated in the Y column were independently obtained, it is a comforting arithmetic exercise to note that their values check well with the summated exponents of appropriate cross products, *cf.* $Y_1 \cdot Y_2 = Y_3$; $m + b_2 = k_i = 0.72$, or for $Y_3 \cdot Y_4 = Y_6$, $k_i + k_j = k_{ij} = 0.774$.

Of special interest here is the fact that the slopes of Y_3 and Y_4, representing, respectively, total number and mass of mitochondria per liver, fall on either side of the value $k = 0.73$ relating total body metabolism to body weight. It is possibly significant that the slope for number ($k = 0.722 \pm .014$) is considerably closer to 0.73 than is the value for mass. With the present statistics, however, it can be stated only that since the difference between the respective k values for Y_3 and Y_4 is not quite significant, certainly their values are not distinguishable from an intermediate value of similar variance.

Other comparisons of k values on liver metabolism against body weight are available from the data. Thus the product QO_2 of homogenate (Y_7) and liver weight (Y_1) gives

$$k = b + m = 0.675 \pm .017,$$

while an oxygen utilization value (Y_{11}) derived from O_2 consumed per mitochondrion, taken from data on oxygen exchange of the *M* fraction and multiplied by the count of mitochondria per gm. liver, gives with liver weight the slope:

$$k = 0.695 \pm .0186$$

Both of these agree precisely with the $k = 0.697$, denoted by Y_{10} in the table.

It is relevant to note that these values of k appear entirely consonant with those which can be derived by comparable treatment of the available published data on QO_2 of liver slice and liver organ weight regression on body size. The m value for liver is available from Brody (1945) as 0.867 ± 0.009 for fresh weight in mature mammals. Using this and the slopes of QO_2 data cited earlier, we get with Kleiber's data, $k = 0.867 - 0.228 = 0.639$ and, with Kreb's, $k = 0.867 - 0.115 = 0.752$, while the same slope for liver weights, with the present QO_2 of homogenate, would give $k = 0.867 - 0.145 = 0.722$.

A final note appears warranted on the function, Y_4 (TABLE 3), denoting the logarithmic regression of apparent mitochondrial mass on body weight. This slope (0.0527) is small but significantly positive and, as such provides a basis for the calculation of a theoretical (though fairly speculative) surface and linear dimension of the mitochondria of the respective species. The product of such a unit surface by the number of mitochondria in the liver would then give the total mitochondrial surface per liver. Its value would depend, of course, both on number and mass of mitochondria and, hence, should correlate well with oxygen utilization.

From the data, we have the apparent mass of the mitochondrion given both in absolute terms and as a function of body weight. If the shape of the mitochondrion is assumed to be spherical and the density given, e.g. of the order of 1.05 (arbitrarily chosen), the mitochondrial surface may be calculated from its relation to volume. Thus if volume = mass/density, and surface $\propto$ (volume)$^{2/3}$, then, with the density given as above, the surface becomes

$$S = 4.7(M)^{2/3} \tag{7}$$

where M is mass (mg. $\times\ 10^{-9}$) per mitochondrion. From this may be obtained the apparent surfaces of the mitochondria of the respective species as well as their radii (TABLE 5) and, from the table, it is evident that the mass difference of about 35 per cent from rat to steer results in a surface increase of about 25 per cent with an increment of radius of only about 13 per cent. The calculated radii appear well within the limits of published data on mitochondrial dimensions.[26]

The aggregate total surface of the mitochondria of the liver should be given by the product of their number times the unit surface. The regression of this total surface against body weight is easily predicted if one assumes, as before, that for the mitochondrion

$$S = c'(M)^{2/3}, \tag{7a}$$

and, empirically, from the data (TABLE 3), one then selects the regression of M on body weight, *i.e.*,

$$Y_4 = M = aW^{0.0527} \text{ and the number}$$
$$Y_3 = N = a'W^{0.7216}$$

TABLE 5

MITOCHONDRIAL DIMENSIONS COMPUTED FROM APPARENT DRY MASS, AND FROM ESTIMATED WET MASS ASSUMING 80 PER CENT WATER CONTENT

Species	*Mass (mg. × 10^9)*	*Surface (μ^2)**	*Radius (μ)**
Rat	0.721	3.78	0.547
	3.605	10.84	0.930
Rabbit	0.793	4.05	0.568
	3.965	11.78	0.968
Sheep	0.948	4.40	0.595
	4.740	13.25	1.025
Steer	1.079	4.95	0.628
	5.395	14.46	1.072

*Calculated values, assuming spherical shape and constant density = 1.05 gm. cm.$^{-3}$; if a density of 1.30 were assumed, the calculated surface would be reduced 13 per cent and the radius by about 3.5 per cent.

one obtains by substitution the total surface S_N of the liver mitochondria as

$$\begin{aligned} S_N &= c' M^{2/3} \quad a'W^{0.722} \\ &= c'''W^{0.757} \end{aligned} \tag{8}$$

The value 0.757, derived for the exponent in EQUATION No. 8, finds no special significance in the present set of k values. While it necessarily falls within the range set by its constituent parameters, mass and number, it does not appear to improve the prediction of QO_2 obtained from the latter. With the allometric constant, m = 0.8196, obtained for liver in the present study, a surface-governed metabolic pattern would correspond aerobically to a QO_2 giving b = –0.063, which is significantly below the experimental values. Used with Brody's m = 0.867, however, one would expect the slope of QO_2 on this basis to give b = –0.110, a value near that given for Kreb's liver data and also somewhat closer to those reported here.

Summary

(1) The current study has demonstrated in a series of mammals, including rat, rabbit, sheep, and steer that, with increasing size of the species, the number of mitochondria per gram of liver decreases, while the apparent individual mass of the mitochondrion is somewhat increased. The product of total number times mass per gram is not a constant but, rather, it increases with the 0.77 power of the body weight. Thus the total quantity of mitochondrial substance is relatively smaller in the steer than in the rat, with respect both to the size of the liver and the total size of the animal.

(2) The oxygen utilization of the liver homogenate is shown statistically to depend more directly upon total number of mitochondria present per gram than upon the unit mass of the mitochondrion.

(3) The regression of oxygen utilization of total liver on body weight appears to fall well within the range of regressions derived from published data when treated by similar methods.

(4) The aggregate mitochondrial surface, as computed from data on unit mass and number of mitochondria in the liver, gives a regression against total body weight that reflects its constituent parameters in being grossly similar to the coefficient obtained for oxygen utilization.

(5) The evidence available appears to establish beyond reasonable doubt that, at least in mammalian liver, the mitochondrial amount bears essentially the same quantitative relation to total metabolism as to total body size, and it appears probable that the relative amounts of these elements in any given tissue will prove the controlling factor in

determining the regression of oxygen utilization on total body size of the species.

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